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Research Article

Response of different wheat varieties to *Bipolaris sorokiniana* at seedling stage under laboratory condition

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ABSTRACT

Spot blotch (*Bipolaris sorokiniana*) is a major disease of wheat in warm, humid wheat-growing regions. The aim of present study was to evaluate the infection symptomology and disease severity to different wheat varieties under laboratory condition. A total of ten wheat genotypes including RR-21 (a susceptible check) and Aditya (a resistant check), were evaluated for seedling stage resistance to spot blotch disease through artificial inoculation under laboratory condition at Gokuleswor, Baitadi during 2018. Based on disease severity and area under disease progress curve (AUDPC), Aditya was found resistant and RR-21 was found susceptible The tested genotypes were categorized on the basis of total AUDPC value, where Banganga, BL4341, BL4407 and local genotype were moderately resistant, BL-4406, NL-1190, NL-297 and NL-971 were found moderately susceptible to spot blot at seedling stage. The susceptible check, RR-21 had the highest disease severity (44%) as well as mean AUDPC value (51.38).

Keywords: Area under disease progress curve, disease severity, *Bipolaris sorokiniana,* spot blotch disease

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INTRODUCTION

Wheat is one of the major cereal crop of Nepal. It has been grown in 706843 ha area and has the productivity of 2.75 MT/ha (CBS, 2018/19). *Helminthosporium* leaf blight (HLB) is one of the major disease of wheat of warmer, non-traditional wheat growing areas of South Asia. HLB is the complex of spot blotch i.e. *Bipolaris sorokiniana,* Sacc. and tanspot i.e. *Pyrenophora tritici repentis* , Died. The disease became important after the green revolution though it has been present for many years (Devkota, 1993). In Nepal, HLB was very severe in Terai areas. Rice-wheat cropping system which is widely practiced in South Asia provides a favorable environment for the survival and multiplication of foliar blight pathogens because rice serves as a host species for the spot blotch and tan spot fungi and rice stubbles also play a role of crop residue to harbor the fungi after rice harvest (Saari, 1998; Mishra, 1973).

B. sorokiniana can cause seedling blight, head blight, leaf blotch, leaf spot, leaf blight, foot rot, discolored grain, black pointed grain and also results in sterile spikes if the infection is severe (Mitra 1931). The disease cause serious problem in wheat production all over the world (Duveiller & Glchrist 1994). The loss of 20% and more had been recorded due to spot blotch in South-Asia (Saari, 1997) and as high as 85% under favorable disease condition (Raemakers, 1987).

The combined effects of high temperature, high relative humidity and long period greater than 12 hours of leaf wetness caused by rainfall, and dew are conducive to foliar blight development in the Indo-gangetic Plains where wheat is grown from November to April (Dueviller, 2004). Higher level of conidial spread of tan spot occurred in February and spot blotch became predominant only in the latter crop season as temperature increased and wheat maturity progressed in Nepal (Dubin & Bimb, 1994).

The pathogen is seed borne and seed transmitted in nature (Bazlur Rashid, 1998) and may exist in different part of the seed. Seed borne source infected with *Bipolaris sorokiniana* cause seedling infection during the early crop stage. Seedlings or young plants become infected at the roots, crown or other below ground parts. Seedling disease is favored by stress factors such as planting in hot, dry soils. The loss due to the root rot was about 14% (Saari, 1997). In Nepal the seed infection was found between 5%-89.1% and the germination of seed ranged from 33.7-94% (Shrestha et al, 1997). Though the loss incurred from seedling infection is not very high but high level of infected seed sowing may cause seedling death and crown root rot (CRR) which is caused by different soil borne fungal complex like *Bipolaris, Fusarium* etc. The yield loss due to CRR may range from 6% to 44% depending on cultivar, planting date, location (Verma *et al*., 1974; Diehl *et al*., 1983). Under severe condition spot blotch causes 100% yield loss (Mehta, 1994).

In Nepal wheat is mostly grown from November to April generally in the residual moisture of the rice crop. Rainfall received during this time of the year is generally very low and rainfed farming system is under practice. So, in conditions wheat is conducive to drought which may result heat stress to the plants. Terminal heat stress increases the severity of spot blotch (Sharma & Duveiller, 2004). Drought is an expanding and creeping threat of world slowly taking hold of an area and tightening its grip with time (Mishra *et al*., 2002). Due to the climate change factors the rainfall pattern has shifted to a later date. This has caused the shift of rice cropping season to later date as well. Hence farmers are compelled to sow wheat late in the season. At this period of year temperature gradually rises making the conditions

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conducive to spot blotch infection. Furthermore, heat stress during that period of time combines with spot blotch to increase severity of the disease to cause a high loss in the yield.

Spot blotch and root rot resistant varieties are needed for tropical environment of Nepal for efficient and stable wheat production (Singlton, 1988). Combined resistance to seed infection, root rot and spot blotch was not identified in any one genotype (Bhandari, 2001), indicating the resistance in different parts of wheat crop may be governed by different genes. The information on response of different wheat genotypes to the spot blotch would be very helpful in breeding for resistance against the disease.

MATERIALS AND METHODS

Preparation of Growth medium

Potato Dextrose Agar (PDA) medium was prepared by by mixing 9.75g PDA with 250ml of water. The media was sterilized by autoclaving at 15 psi and 121˚C for 2 hours and poured in sterilized test tubes. The tubes were kept in slanting position and were then allowed to cool and solidify the slants.

Preparation of Pure culture

Pure culture of the fungus *Bipolaris sorokiniana* was prepared by picking single spore from the fungal colony growing on the surface of the seeds placed in wet blotting paper with the help of inoculating needle and placing them on the PDA test tubes. This process was carried out in completely aseptic condition to prevent contamination of the medium with other microorganisms. The tubes were well plugged with non-absorbent cotton and incubated at room temperature of 25^{o}C in an incubator. The tubes were checked regularly for the growth of the mycelium of the fungus. Within 12-15 days, 30 culture tubes were successfully prepared with pure culture of the fungus.

Sowing of the wheat genotypes

Plastic cups of 3 inch diameter and 4 inches height were used for sowing wheat seeds. Humus rich top soil was mixed with well decomposed farm yard manure (FYM) at 3:2 ratio. The mixture was then made free of stones, brick pieces, plastics, and other such materials. Soil was sterilized with 1% formaldehyde solution, covered with plastic sheet for two days. Working room was also fumigated with formaldehyde and potassium permagnate solution. Then the cups were filled with the sterilized soil media.
fifteen seeds were sown in each cup at a depth of 2-3 cm. The moisture content was maintained at around field capacity in the soil during sowing. Sowing was carried out on 18th Jan 2018 and irrigated on seventh day after sowing.

Experimental set up

The experiment was laid out in Complete Randomized Design (CRD) with four replications at the plant pathology laboratory of Gokuleshwor Agriculture and Animal Science College, Baitadi. Ten wheat genotypes were sown in plastic cup filled with sterilized soil:FYM mix.

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Table 1: Wheat genotype and their source

SN	Wheat Genotype	Source
1	Aditya	NWRP, Bhairahawa
2	Banganga	NWRP, Bhairahawa
3	BL4341	NWRP, Bhairahawa
4	BL4406	NWRP, Bhairahawa
5	BL4407	NWRP, Bhairahawa
6	Local	Local farmer
7	NL1190	NWRP, Bhairahawa
8	NL297	NWRP, Bhairahawa
9	NL971	NWRP, Bhairahawa
10	RR-21	NWRP, Bhairahawa

Mass culture

Mass culture was prepared on 8th Feb 2018 in completely aseptic condition. The PDA media was poured in the petri-plates @ 20 ml/plates and a small 1g of bactericide (Sterptomycine) was added in the media when its temperature was around 50˚C to prevent bacterial contamination. Each petri-plates were then inoculated with the mycelium from the pure culture at five places approximately equidistant from each other to ensure uniform growth of the fungus. The inoculated petri-plates were placed in an incubator at room temperature.

Preparation of the inoculums and inoculation

Mass culture was ready to prepare the inoculum on 12th Feb 2018. The petri-plates covered by the pure culture of *Bipolaris sorokiniana was* scrupped with the edge of glass-slide and distilled water was added. The spores thus washed off were collected in a sterilized conical flask. To remove the mycelia growth, the spore suspension was passed through muslin cloth. The suspension of spores in distilled water collected from each petri-plate was checked under the microscope for concentration of spores. The spore suspension in the distilled water was maintained at around 50000 spores/ml by using Haemocytometer and the final volume of the suspension was made 200 ml.

The wheat seedlings were inoculated at two-leaf stage on 30th Magh by spraying the spore suspension at evening time. Each cup with the seedlings was sprayed uniformly with the help of atomizer.

Management of the inoculated seedlings:

Immediately after the inoculation the cages with the cups containing the seedlings were completely covered with jute sacks soaked in water to maintain high humidity required for the germination of fungus and also for penetration of the host by the germ tube. The jute sacks were watered two times at an interval of 12 hours. The jute sacks were then removed after 24 hours of inoculation.

Disease assessment

Assessment of disease was done by single digit scoring. Scoring was done four times i.e. 2, 4, 6 and 8 days after inoculation on 17th, 19th, 21st and 23rd February respectively. At the time of scoring the seedlings of all the wheat genotypes were at four leaves stage.

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Disease scoring

Scoring was done visually on the basis of infected leaf area of the seedlings. Two plants were randomly selected from first and second replication and one plant was randomly selected from the third replication of each genotypes. Disease scoring was done on the basis of standard diagram developed by CIMMYT (Muzeeb-Kaazi *et al*., 1996).

Table 2: Disease scoring of the spot blotch on wheat seedlings at four leaved stage

Score	Infected leaf area %	Amount of Disease
0	0	No visual spots on all the four leaves
1	10	1-2 spots on the four leaves
2	20	3-4 spots on the four leaves
3	30	5-6 spots on the four leaves
4	40	One leaf completely diseased with spots and 1-2 spots on the remaining three leaves
5	50	One leaf completely diseased with spots and 3-5 spots on three leaves
6	60	Two leaves completely diseased with spots and 1-2 spots on the other two leaves
7	70	Two leaves completely diseased with spots and 3-5 spots on the other two leaves
8	80	Three leaves completely diseased with spots and few spots on the remaining leaf
9	90	All four leaves completely diseased with spots and plant dead

Disease Severity

On the basis of the single digit scores on the four dates, disease severity of the wheat genotypes were calculated using following formula

$$DS = \frac{\text{Sum of all numerical ratings} \times 100}{\text{Number of sample plants} \times \text{highest rating}}$$

Estimation of AUDPC

The area under disease progress curve (AUDPC) was calculated by summarizing the progress of disease severity. Three AUDPC values from single digit scoring on the four dates were calculated by using the following formula given by Das *et al*. (1992) and Shrestha et al. (2019)..

$$AUDPC = \sum_{i=1}^{n-1}\left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where,
Yi= disease scored on 1th first date
Ti= date on which the disease was scored

n = Number of dates on which disease was scored
Mean AUDPC was calculated by taking the average of the three AUDPCs i.e. AUDPC 1, AUDPC 2 and AUDPC 3.

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Statistical analysis

All the recorded data were subjected to analysis of variance using R-Studio and the treatment means were compared by the Least Significant Difference (LSD) test at 5% level (Gomez & Gomez, 1984; Shrestha, 2019). A simple correlation and regression analysis was performed between the selected parameters. Microsoft excel was used for data entry, tabulation and graphs.

RESULTS AND DISCUSSION

Disease Severity (%)

Analysis of variance (ANOVA) showed highly significant difference between genotypes and disease severity at 2, 4, 6 and 8 days after inoculation (DAI) (Table 1). At 2 DAI, disease severity lies in the range of 0.00%-7.77%. Lowest disease severity was observed in Adaitya, BL-4341 and NL971 (0.00%). The highest disease severity was recorded on RR-21(7.77%). At 4 DAI, the lowest disease severity was observed in Aditya (2.77%). The highest disease severity was recorded on RR-21(20.55%). At 6 DAI, the lowest disease severity was again seen in Aditya (3.61%). The highest disease severity was seen in RR21 (30.55%). At 8 DAI, the lowest disease severity was again seen in Aditya (5.00%). The highest disease severity was seen in RR21 (44.16%).

Table 3: Spot blotch disease severity (%) of ten wheat genotypes at 2, 4, 6, 8 days after inoculation (DAI)

	Disease severity on			
Genotypes	2 DAI	4 DAI	6 DAI	8 DAI
Aditya	0.00^{d}	2.77^{e}	3.61^{c}	5.00^{c}
Banganga	1.11^{c}	8.05^{cd}	17.50^{b}	26.94^{b}
BL-4341	0.00^{d}	8.05^{cd}	16.94^{b}	28.88^{b}
BL-4406	1.11^{c}	9.44^{bcd}	16.94^{b}	27.22^{b}
BL-4407	0.00^{d}	6.66^{d}	17.50^{b}	28.88^{b}
Local	0.00^{d}	7.77^{cd}	16.94^{b}	29.72^{b}
NL-1190	3.05^{b}	10.55^{bc}	18.61^{b}	30.27^{b}
NL-297	1.11^{c}	11.11^{b}	21.38^{b}	31.66^{b}
NL-971	0.00^{d}	10.00^{bc}	20.27^{b}	31.94^{b}
RR-21	7.77^{a}	20.55^{a}	30.55^{a}	44.16^{a}
Mean	1.41	9.5	18.03	28.47
CV	51.13	20.65	18.62	16.75
SEM(±)	0.51	1.39	2.41	3.37
LSD	1.04	2.83	4.85	6.89
P-value	5.02^{e-15}	$9.17e^{-11}$	$6.32e^{-09}$	$3.03e^{-09}$

(Figures followed by the same letters in a column are not significantly different by LSD at 5% level of probability)

Pandey et al. (2018) also found RR-21 with highest disease severity value throughout the wheat crop period in national wheat research program, Bhairahwa, Rupandehi. Aryal (2013) reported that the disease severity in Aditya was low as it had 6.25% seed infection and 2.43% and 1.513 % seedling incidence in first and second date of sowing, respectively.

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Area under disease progressive curve (AUDPC)

Analysis of variance (ANOVA) showed highly significant difference between genotypes for AUDPC-1, AUDPC-2 and AUDPC-3 (Table-2). AUDPC-1 lies in the range of 2.77-28.33. Lowest AUDPC-1 was seen in Aditya (2.77) and highest AUDPC-1 was seen in RR21 (28.33). AUDPC-2 lies in the range of 6.38-51.11. Lowest AUDPC-2 was seen in Aditya (6.38) and highest AUDPC-2 was seen in RR-21 (51.11). AUDPC-3 lies in the range of 8.61-74.72. Lowest AUDPC-3 was seen in Aditya (8.61) and highest AUDPC-3 was seen in RR21 (74.72). Mean AUDPC lies in the range of 5.92-51.38. Lowest Mean AUDPC was seen in Aditya (5.92). But among all the tested genotypes most severe genotype was RR-21 with mean AUDPC of 51.38. Lower AUDPC value in Aditya variety was also reported by Aryal, 2013 when screening 20 genotypes against spot blotch. Pandey et al. (2018) also found RR-21 with highest AUDPC value throughout the wheat crop period in national wheat research program, Bhairahwa, Rupandehi.

Table 4: Spot blotch AUDPC value of ten wheat genotypes at 2, 4, 6, 8 days after inoculation (DAI)

	AUDPC VALUE				
Genotypes	AUDPC1	AUDPC2	AUDPC3	TOTAL AUDPC	MEAN AUDPC
Aditya	2.77^{f}	6.38^{d}	8.61^{c}	17.77^{d}	5.92^{d}
Banganga	9.16^{cde}	25.55^{bc}	44.44^{b}	79.16^{bc}	26.38^{bc}
BL-4341	8.05^{de}	25.00^{c}	45.83^{b}	78.88^{bc}	26.29^{bc}
BL-4406	10.55^{bcd}	26.38^{bc}	44.16^{b}	81.11^{bc}	27.03^{bc}
BL-4407	6.66^{e}	24.16^{c}	46.38^{b}	77.22^{c}	25.74^{c}
Local	7.77^{de}	24.72^{c}	46.66^{b}	79.16^{bc}	26.38^{bc}
NL-1190	13.61^{b}	29.16^{bc}	48.88^{b}	91.66^{bc}	30.55^{bc}
NL-297	12.22^{bc}	32.50^{b}	53.05^{b}	97.77^{b}	32.59^{b}
NL-971	10.00^{cd}	30.27^{bc}	52.22^{b}	92.50^{bc}	30.83^{bc}
RR-21	28.33^{a}	51.11^{a}	74.72^{a}	154.16^{a}	51.38^{a}
Mean	10.92	27.53	46.5	84.94	28.31
CV	20.29	17.63	16.77	16.39	16.39
SEM(±)	1.56	3.43	5.51	9.85	3.28
$LSD_{0.05}$	3.2	7.00	11.26	20.11	6.70
P-value	$5.66e^{-14}$	$2.09e^{-10}$	$1.72e^{-09}$	$5.82e^{-11}$	$5.82e^{-11}$

(Figures followed by the same letters in a column are not significantly different by DMRT at 5% level of probability)

Total AUDPC calculated from AUDPC 1, AUDPC 2 and AUDPC 3 of the 10 genotypes reveals Aditya (17.77) as the resistant variety. BL4407 (77.22), BL4341 (78.88), Banganga (79.16) and Local (79.16) were the moderately resistance genotypes. BL4406 (81.11), NL1190 (91.66), NL971 (92.50) and NL297 (97.77) were moderately susceptible genotypes. RR-21(154.16) was susceptible genotype. Lowest total AUDPC value in Aditya was also obtained by Aryal (2013) when the genotype was sown in normal and late condition. This result is also comparable with mean AUDPC value of 83.33 and 130.56 of Aditya and RR-21 (Dhakal, 2016).

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Table 3: Categories of 10 wheat genotypes on the basis Total AUDPC values

Category	Range	Total AUDPC
Resistant	0-40	Aditya
Moderately Resistant	41-80	Banganga
		BL-4341
		BL-4407
		Local
Moderately Susceptible	81-120	BL-4406
		NL-1190
		NL-297
		NL-971
Susceptible	121-160	RR-21
Highly Susceptible	>160	-

CONCLUSION

Spot blotch is a major fungal disease of wheat caused by *Bipolaris sorokiniana.* A lab experiment was conducted to determine the response of different varieties of wheat to *Bipolaris sorokiniana* under laboratory condition at seedling stage. Among ten different wheat genotypes evaluated in Complete Randomized Design (CRD) the highest disease severity and AUDPC value was recorded for RR-21 while it was lowest for Aditya, the susceptible and resistant checks, respectively. Categorization of ten genotypes on the basis of total AUDPC value revealed Aditya as the resistant variety; Banganga, BL4341, BL4407 and Local were moderately resistant; while BL4406, NL1190, NL297 and NL971 were moderately susceptible to spot blot disease at seedling stage. The identified wheat genotypes could be further used in breeding programs.

Author Contributions

D.D. and N. D. had their contribution in report writing and analyzing along with supporting hands from H.G. and S.B.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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